**Original Paper**

# Sutdy on the Special Components of Rice Bran Oil (II)

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**Synopsis:** Unsaponifiable matters of rice bran oil, especially the constituent of oryzanol, ferulic acid was researched. The Super Oxide Dismutase (SOD) effect of ferulic acid in edible rice bran oil was studied. As the result in vivo test it showed that active oxygen eliminating effect was more distinct at low concentration range of ferulic acid than Vitamin E. It may be suitable to develope healthfood, physiologically active food and pharmaceutical.

## 1. Introduction

In the former report,[1] triterpene alcohol as the component of oryzanol was discussed, and here in the present paper another constituent of this ester i.e. ferulic acid is studied. R. Kaneko and T. Tsuchiya[2),10)] discovered oryzanol in rice bran oil, and defined it as a materi ' which had near 231, 291 and 315 nm UV absorption max, and which was physiologically a tive. However those absorption bands correspond to a conjugate double bond of ferulic acid, which was studied in the same decade.

Ferulic acid is the basic component of lignin and finally cellulose, and widely distributes in plant, especially Oryza Sativa Linne group. **Fig. 1** shows biosynthesis of it by photosynthesis, metabolism or enzyme reaction.[3)] Furthermore **Fig. 2a~b** show the similarity of UV absorption patterns of isomers of ferulic acid and oryzanol i.e. ferulic ester of triterpenol. **Fig. 3** shows HPLC pattern of ferulic acid.

In this paper one of the authors H. Naruse researched SOD effect of free ferulic acid. SOD means the Super Oxide Dismutase, a kind of useful enzyme which deactivates biologically harmful superoxides.

## 2. Basic properties of Ferulic Acid

The physical and chemical properties of ferulic acid were reserched already as follows, and here the authors research biotechnologically. The industrial production process of this matter started from rice bran oil was researched by T. Tsuno et al. and they developed industrial manufacturing method and have registered several Japan Patents[6)] recently.

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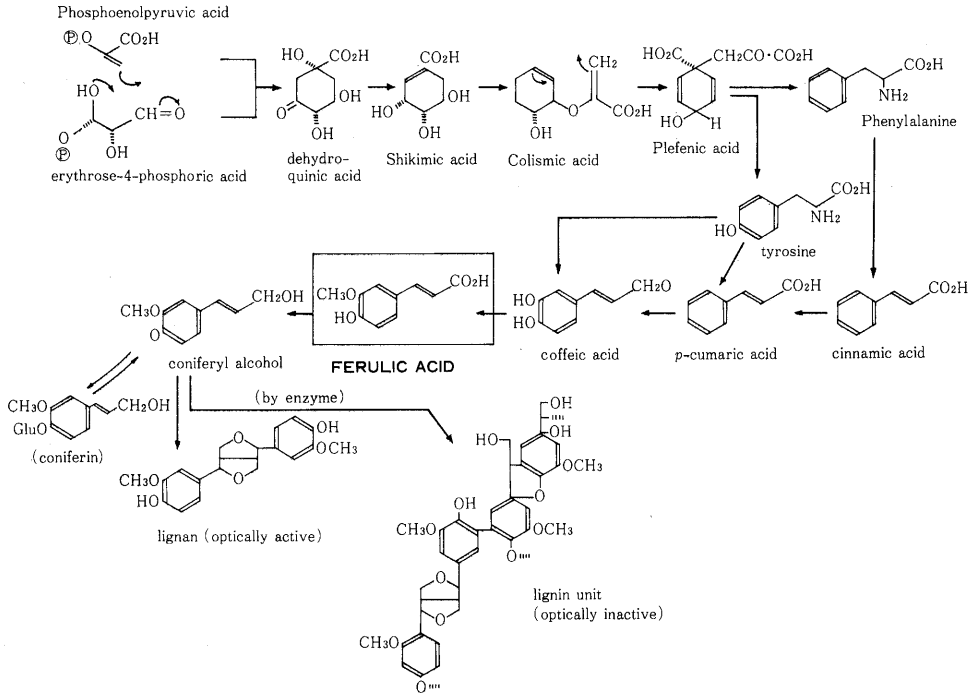



**Fig. 1** Biosynthesis of Ferulic acid.[3]

## 2.1 Physical and Chemical Properties of Ferulic Acid[7]

Ferulic acid is chemically 4-hydroxy-3-methoxy-cinnamic acid; 3-methoxy-4-hydroxy-cinnamic acid; 3-(4-hydroxy-3-methoxyphenyl)propenoic acid. $C_{10}H_{10}O_4$: mol wt. 194.18. C 61.85%, H 5.19%, O 32.96%, mp 169°C; Soluble in hot water, ethanol, ethyl acetate and acetic acid. Moderately soulble in ethyl ether. Sparingly soluble in petr. ether, benzene; Forms sodium salt; Ethyl ester, $C_{12}H_{14}O_4$, crystals from petr. ether. mp 38°C; Monohydrate, $C_{12}H_{14}O_4H_2$ O, crystals.

*cis*-form; Yellow oil, absorption max. in ethanol; 316 nm.

*trans*-form; Orthorhombic needles from water, mp 174°C; absorption max in ethanol; 236, 322 nm.[8] It isomerises to *cis*-form, but keeps equilibrium.

## 2.2 SOD Effect of Ferulic Acid

"Oxidative Stress" starts from a life body tending to an oxidation.

"Active oxygen" causes many harmful effects for the life body (Inflammation, Aging, Cancer, effect and poison of medicines, infection of vermin and microorganism).

The question is "How protect against this harm". It made many studies on SOD in the life body protect this active oxygen. Generally, in the case of medicine, low molecular compounds have SOD function more utilizable than SOD from an enzyme source. Because an enzyme has some defect such as destability, antibody formation impermeability through a menbrane for large molecules, metabolism in life body is quickly from an essential property of protein.

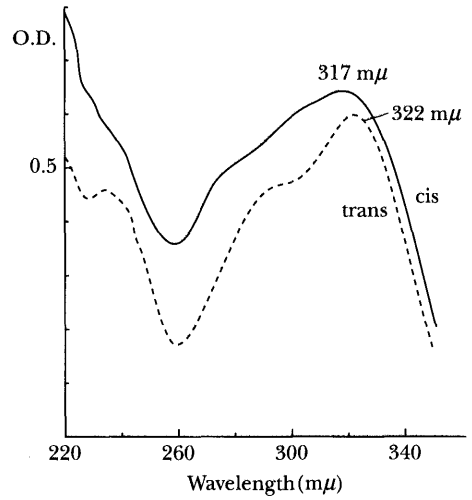



**Fig. 2-a** UV Abs. Spectrum of *cis-* & *trans-* Ferulic Acid (Et. OH soln.)[4]

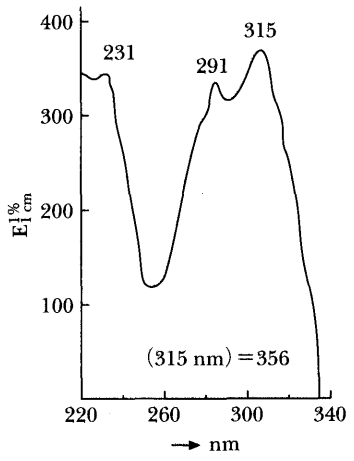



**Fig. 2-b** UV Abs. Spect. of oryzanol (by Y. Takeshita)[5]

Low molecular compound having SOD function is "Vitamin E", "polyphenol". In this reserch, as a bioactive material of SOD function "Ferulic acid" in vivo test was tried.

## 3. Materials and Methods

### 3.1 Animal and Management

Wister rats (male, weeks age) were purchased from Japan SLC (shizuoka, Japan). The rats were put in an amimal room at 12 H of light and darkness, and at 12 times/H of arouse alternately.

The solid ration and water were fed at any time. The rats were bred in a group of 6 in a plastic cage for about one week.

### 3.2 Materials

Ferulic acid···Available from Tsuno Fine Chemical Co., (Wakayama, Japan) and its purity was 99.4%. (cf. **Fig. 3** HPLC pattern).

Polyphenol···The commodity name; (Tea, SANFENON 100S) was supplied by TAIYO KAGAKU Co., (Mie, Japan)

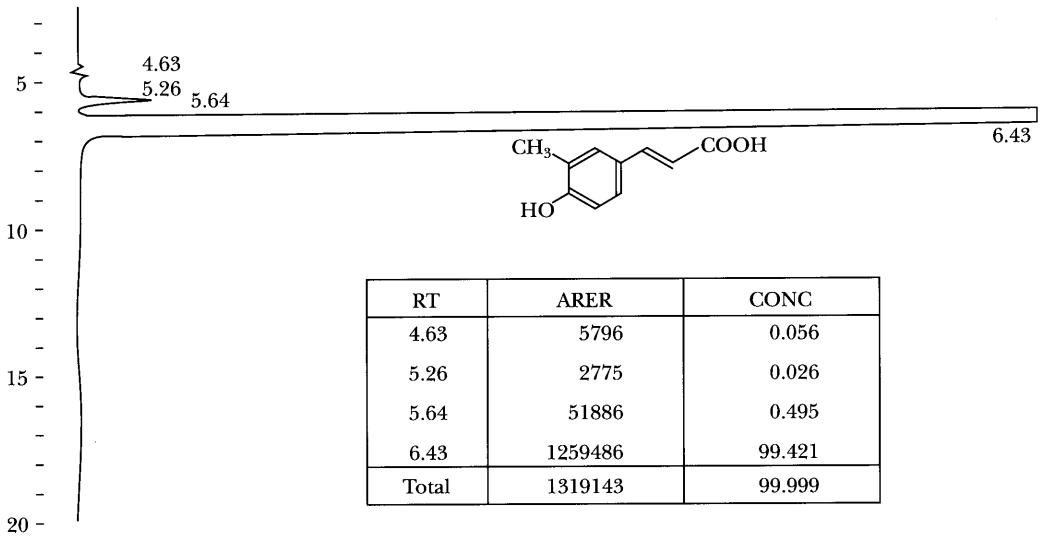
Vitamin E ···It is dl-α-tocopherol, purchased from NIPPON ROCHE Co., (Tokyo, Japan) and its purity was 50%.

### 3.3 Experimental method of Materials

Ferulic acid, Polyphenol and Vitamin E were prepared as powdery food containing 0.5, 1.0, 2.0 wt. % ingredient respectively, and each rat was freely fed on these mixed food and water.

### 3.4 Measurement of body weigt and blood plasma

Measurement was tried at every 1 week for 4 weeks, but Uric acid and Vitamin C were




| RT | ARER | CONC |
|---|---|---|
| 4.63 | 5796 | 0.056 |
| 5.26 | 2775 | 0.026 |
| 5.64 | 51886 | 0.495 |
| 6.43 | 1259486 | 99.421 |
| Total | 1319143 | 99.999 |

**Condition**
sample: Ferulic acid; Column; Lichrosorb SI. 100−1; Column Size: 4.0 mm$\phi$×250 mm.
Mobile Phase; n-Hexane: etyl acetate: Ethanol (30 : 30 : 2)+0.1% Phosphoric acid.
Pressure; 8 kg/cm$^2$; Flow Rate: 1.0 mL/min; Column temp: Room temp.
Detector: UV 300 nm; Chart speed: 0.5 cm/min; Range: 0.1; Sample size; 2 $\theta$L (10 mg/10 mL)

**Fig. 3** HPLC pattern of Ferulic acid

tried at 4th week only. The measurement was tried about the body weight and TBRS in blood plasma, and about SOD, uric acid and vitamin C in blood.

## 4. Results

**4.1** The result of body weight of the rats is shown in **Table 2.**
**4.2** The result of SOD of the rats is shown in **Table 3 & Fig. 4.**
**4.3** The result of TBRS of the rats is shown in **Table 4.**
**4.4** The results of Uric acid and Vitamin C of the rats are shown in **Table 5.**

## 5. Discussion

① The SOD activity of Vitamin E showed a remarkable effect, but those of polyphenols of Tea and Ferulic acid showed no remarkable effects. It is thought to unusually rise at 4th week of vitamin E.
② Ferulic acid tends to get rid of "Active Oxygen" and supports "Homeostasis" as a result of SOD active, TBRS and uric acid of last test.
③ Ferulic acid tends to strongly show an active oxygen reducing effect at low concentration than vitamin E.
④ At ferulic acid supplied, blood Vitamin C showed tendency of decrease. An excess vitamin C was on the decrease at supplied antioxidant, by reason of a biosynthetic

**Table 2** Body weight from 0~4 th weeks

(g/head)

| Period(week) | | 0 | | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample \ Data | | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. |
| Control | | 181.83 | 1.94 | 222.50 | 5.86 | 247.33 | 8.29 | 272.33 | 9.33 | 278.50 | 5.36 |
| Ferulic a. | 0.5% | 180.50 | 4.04 | 220.50 | 6.95 | 247.83 | 8.57 | 269.83 | 6.18 | 279.67 | 5.89 |
| Ferulic a. | 1.0% | 180.17 | 5.27 | 221.83 | 4.26 | 253.50 | 8.24 | 272.17 | 3.13 | 284.17 | 4.75 |
| Ferulic a. | 2.0% | 180.83 | 3.97 | 221.33 | 4.08 | 249.00 | 6.51 | 270.17 | 6.79 | 280.83 | 6.85 |
| Polyphenol | 0.5% | 180.50 | 4.04 | 221.33 | 3.93 | 253.00 | 6.69 | 269.00 | 10.06 | 281.17 | 9.37 |
| Polyphenol | 1.0% | 179.67 | 6.65 | 221.33 | 4.59 | 250.33 | 4.23 | 271.33 | 5.20 | 282.00 | 6.36 |
| Polyphenol | 2.0% | 180.50 | 3.78 | 222.50 | 3.15 | 251.83 | 5.88 | 270.67 | 2.07 | 284.50 | 4.76 |
| Vitamin E | 0.5% | 182.50 | 4.97 | 223.00 | 4.00 | 252.83 | 4.83 | 273.67 | 9.20 | 284.33 | 6.15 |
| Vitamin E | 1.0% | 177.83 | 5.04 | 219.33 | 4.89 | 251.33 | 8.33 | 272.33 | 8.14 | 284.33 | 4.80 |
| Vitamin E | 2.0% | 181.17 | 3.19 | 219.83 | 8.52 | 253.83 | 7.65 | 274.00 | 10.86 | 281.67 | 2.34 |

In the former or following tables and figures listing results, "Aver." and "S.D." show "average value" and "standard deviation" respectively.

**Table 3** SOD from 0~4 th weeks

(units/m*l* blood)

| No | Period(week) | | 0 | | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample \ Data | | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. |
| 1 | Control | | 1827 | 420 | 1375 | 225 | 1372 | 113 | 1675 | 421 | 1582 | 239 |
| 2 | Ferulic a. | 0.5% | 1732 | 255 | 1093 | 50 | 1257 | 92 | 2086 | 763 | 2523 | 552 |
| 3 | Ferulic a. | 1.0% | 1589 | 250 | 1157 | 108 | 1096 | 81 | 1747 | 408 | 1601 | 105 |
| 4 | Ferulic a. | 2.0% | 2035 | 444 | 1300 | 90 | 1157 | 151 | 2345 | 441 | 2046 | 45 |
| 5 | Polyphenol | 0.5% | 1757 | 333 | 1392 | 289 | 1121 | 61 | 1822 | 263 | 2563 | 792 |
| 6 | Polyphenol | 1.0% | 1347 | 186 | 1123 | 58 | 1086 | 106 | 1816 | 370 | 2841 | 393 |
| 7 | Polyphenol | 2.0% | 1646 | 95 | 1047 | 78 | 1056 | 37 | 1777 | 248 | 2500 | 254 |
| 8 | Vitamin E | 0.5% | 1743 | 198 | 1039 | 92 | 1066 | 99 | 1655 | 104 | 3825 | 701 |
| 9 | Vitamin E | 1.0% | 1534 | 283 | 1049 | 50 | 987 | 80 | 2327 | 288 | 5589 | 3629 |
| 10 | Vitamin E | 2.0% | 1295 | 39 | 1001 | 95 | 1043 | 86 | 2073 | 306 | 7118 | 2507 |

function of vitamin C of the rats.

⑤ An antioxidant of ferulic acid, polyphenols and Vitamin E had no effect on change of body weight.

Ferulic acid tends to scavenge of "Active Oxygen" at low concentration comparing to vitamin E, but the authors have in mind to need more extend the supply period, by reason of SOD activity decreased age together.

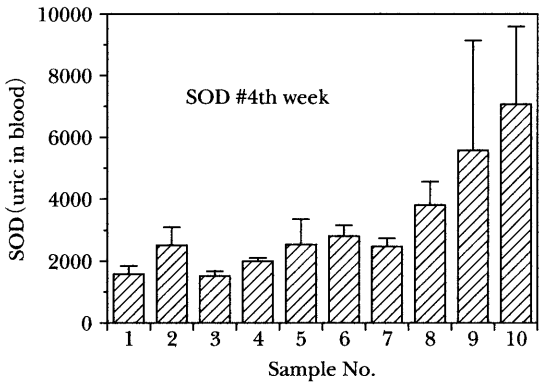



**Fig. 4** SOD Test

**Table 4** TBRS from 0~4 th weeks

(n mol/m/ blood plasma)

| Period(week) | | 0 | | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample \ Data | | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. | Aver. | S.D. |
| Control | | 6.03 | 0.52 | 5.15 | 0.94 | 2.83 | 0.08 | 4.37 | 1.17 | 4.43 | 0.89 |
| Ferulic a. | 0.5% | 6.53 | 1.30 | 4.63 | 0.38 | 3.28 | 0.26 | 4.25 | 0.24 | 3.68 | 1.07 |
| Ferulic a. | 1.0% | 6.12 | 0.83 | 5.13 | 0.63 | 3.25 | 0.34 | 5.02 | 0.21 | 4.03 | 0.16 |
| Ferulic a. | 2.0% | 5.95 | 1.83 | 4.37 | 0.76 | 3.78 | 0.52 | 5.35 | 0.82 | 5.15 | 2.23 |
| Polyphenol | 0.5% | 5.95 | 0.93 | 4.92 | 0.66 | 3.32 | 0.48 | 4.83 | 0.27 | 4.12 | 0.73 |
| Polyphenol | 1.0% | 5.87 | 0.87 | 4.42 | 0.45 | 3.10 | 0.18 | 4.52 | 0.57 | 3.67 | 0.31 |
| Polyphenol | 2.0% | 5.50 | 1.14 | 5.18 | 0.50 | 3.27 | 0.34 | 5.22 | 0.26 | 4.38 | 0.88 |
| Vitamin E | 0.5% | 6.55 | 1.21 | 3.35 | 0.35 | 2.37 | 0.22 | 4.48 | 0.84 | 2.98 | 0.18 |
| Vitamin E | 1.0% | 5.68 | 0.53 | 4.10 | 0.33 | 2.80 | 0.53 | 5.17 | 0.43 | 2.82 | 0.34 |
| Vitamin E | 2.0% | 5.98 | 0.66 | 3.43 | 0.29 | 2.45 | 0.34 | 4.92 | 0.39 | 3.20 | 0.51 |

## 6. Conclusion

There are several researchs about the use or environmental poison of manufactured or natural existent ferulic acid. However, feruric acid is used as the materials of pharmaceutical, agricultural medicine, cosmetic, pigment or food.

As the conclusion of present research, it was discovered that the usefulness of ferulic acid from rice bran oil for the SOD effect by *in vivo* test of rat. The meterial ferulic acid was recognized as trans-ferulic acid by analytical isolation.

**Table 5** Uric acid and Vitamin C of 4 th week
(mg/dl blood)

| Contents | | Uric acid | | Vitamin C | |
|---|---|---|---|---|---|
| Sample | | Aver. | S.D. | Aver. | S.D. |
| Control | | 3.40 | 0.73 | 0.93 | 0.25 |
| Ferulic a. | 0.5% | 3.03 | 0.21 | 0.80 | 0.43 |
| Ferulic a. | 1.0% | 2.85 | 0.94 | 0.58 | 0.15 |
| Ferulic a. | 2.0% | 3.23 | 1.27 | 0.73 | 0.31 |
| Polyphenol | 0.5% | 4.85 | 0.45 | 1.18 | 0.21 |
| Polyphenol | 1.0% | 2.87 | 0.68 | 0.85 | 0.24 |
| Polyphenol | 2.0% | 3.28 | 0.99 | 1.33 | 0.08 |
| Vitamin E | 0.5% | 4.23 | 1.20 | 1.08 | 0.34 |
| Vitamin E | 1.0% | 3.02 | 0.47 | 0.80 | 0.20 |
| Vitamin E | 2.0% | 3.05 | 0.61 | 0.63 | 0.30 |

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論　文

# 米ぬか油の特殊成分の研究，第2報

## ——オリザノールの成分フェルラ酸のSOD効果について——

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**要旨**：米ぬか油の特殊成分特にオリザノール構成成分であるフェルラ酸の生理作用として，生体内の活性酸素を抑制するSOD様効果が，動物実験の結果から明らかになった。また，その効果は低濃度でビタミンEよりも優れている事が明らかになった。

*連絡担当者